RESPONSE SURFACE MODELS FOR THE EFFECTS OF TEMPERATURE, pH, SODIUM CHLORIDE, AND SODIUM NITRITE ON THE AEROBIC AND ANAEROBIC GROWTH OF *STAPHYLOCOCCUS AUREUS* 196E[1]

ABSTRACT

The effects and interactions of temperature (12–45C), initial pH (4.5–9.0), NaCl (0.5–16.5%), and sodium nitrite (1–200 μg/ml) on the aerobic and anaerobic growth of Staphylococcus aureus *196E were studied using 50 ml portions of Brain Heart Infusion Broth in 250ml unsealed and sealed trypsinizing flasks, respectively. The flasks were inoculated to a level of approximately 10^3 cfu/ml, incubated on a rotary shaker, sampled periodically, and enumerated on Tryptic Soy Agar. Growth curves were generated by fitting the data to the Gompertz function using nonlinear regression analysis. The general growth characteristics of* S. aureus *in response to the five environmental variables were similar to those observed by other investigators including (1) enhanced growth in the presence of oxygen, (3) ability to grow at high sodium chloride concentrations, and (3) dependence of the bacteriostatic activity of sodium nitrite on pH and oxygen availability. Supplemental studies indicated that growth kinetics were independent of inoculum size, which allowed the Gompertz A term to be treated as a constant. However, the maximum population density (MPD) achieved by the cultures was dependent on the independent variables, requiring that it be modeled in addition to the Gompertz B and M terms. The MPD was then used to calculate the Gompertz*

C term. Quadratic and cubic response surface models were generated using various data transformations. Quadratic models using and LN-transformation provided reasonable predictions of the effects of the four variables on the growth kinetics of S. aureus, *and should prove useful for providing initial estimates of the behavior of* S. aureus *in foods.*

INTRODUCTION

Even though new foodborne pathogens are continuously being identified, *Staphylococcus aureus* remains a major cause of food borne illness in the United States. During the period from 1973 to 1987, *S. aureus* was the etiologic agent of 367 of 1869 documented bacterial foodborne outbreaks (19.6%), causing 17,248 of 180,906 cases (15.8%) (Bean and Griffin 1990). *S. aureus* is also a leading cause of food poisoning in Canada (Todd 1992).

A key to control of *S. aureus* and other foodborne pathogens is understanding the factors that influence their growth in foods and manipulating those factors to limit potential risks. However, considering that the primary source of *S. aureus* is food handlers and that it is a relatively hardy species, the microorganism is likely to be introduced into and grow in a wide range of food products. It is unlikely that detailed information on the growth characteristic of *S. aureus* would be acquired from more than a limited number of specific foods. Baird-Parker and Kilsby (1987) concluded that the logical approach for determining the probable behavior of a pathogen in a food is the use of predictive models that estimate the microorganism's response to the primary factors affecting its growth and survival. When one considers the large number of potential food formulations and storage conditions, it is evident that validated mathematical models have the potential to be invaluable tools for rapidly and objectively assessing the relative safety of food products.

The literature of foodborne *S. aureus* is voluminous (Smith *et al.* 1983), but the simultaneous study of the quantitative effects of multiple physical and chemical factors influencing its growth characteristics to develop a predictive models has not been reported. Accordingly, the objective of the current study was to assess the effects and interactions of sodium nitrite, sodium chloride, pH, and temperature on the aerobic and anaerobic growth kinetics of *S. aureus* 196E. The data were then used to develop response surface models that could be used to rapidly predict the microorganism's behavior.

MATERIALS AND METHODS

Microorganism

Staphylococcus aureus 196E was used throughout the study. Stock cultures were maintained in Brain Heart Infusion Broth (BHI) (Difco) at 5C, and transferred

monthly. Starter cultures (50 ml BHI in 250-ml flask) were incubated at 28C for 18 h on a rotary shaker (150 rpm).

Culture Techniques

The microorganism was cultured using a modification of the techniques of Buchanan *et al.* (1989). BHI was supplemented with the appropriate level of NaCl to achieve levels of 0.5–16.5% (W/V), adjusted to the desired pH (4.0–9.0) with HCl or NaOH, and brought up to volume. The medium was dispensed in 50 ml portions to 250-ml trypsinizing flasks with a rubber septum inserted in the sidearm sampling port, and sterilized by autoclaving. Filter-sterilized $NaNO_2$ was added aseptically to achieve levels of 0–200 μg/ml. Unless otherwise specified, each flask was inoculated to a population density of approximately 10^3 mfu/ml. Aerobic flasks were closed with a foam stopper. Anaerobic cultures were flushed with sterile N_2 and sealed with a rubber stopper. The flasks were incubated on rotary shakers (150 rpm) at 12–45C ($\pm$ 0.5C).

At intervals appropriate for the culture conditions, samples were withdrawn through the sidearm septum with a syringe fitted with a hypodermic needle. Samples were surface plated on Tryptic Soy Agar (Difco) using a Spiral Plater (Spiral Systems, Inc.), and incubated for 24 h at 37C. The plates were enumerated using a Spiral Systems Laser Counter (Model 500, Spiral Systems, Inc.).

Experimental Design

The initial experimental design for variable combinations to be tested employed a central composite design. Separate data sets were generated for aerobic and anaerobic conditions. Subsequent iterations of model development and data acquisition employed a fractional factorial design, focusing on those variable combinations needed to enhance model effectiveness.

Curve Fitting and Model Development

Plate counts were transformed to $\log_{10}$ values, and growth curves were generated by fitting the Gompertz function (Gibson *et al.* 1988) to the plate count data using ABACUS, a nonlinear curve-fitting program developed by W. Damert (USDA ARS Eastern Regional Research Center). This program employs a Gauss-Newton interative procedure. Typically, the Gompertz A value was fixed at the experimental value obtained for the 0-h sample. Once generated, the Gompertz parameters were used to calculate the cultures' lag phase duration (LPD), exponential growth rate (EGR), generation time (GT), and maximum population density (MPD) as described previously (Buchanan and Phillips 1990).

Quadratic and cubic polynomial models on temperature, pH, NaCl, and $NaNO_2$ of transformations of the Gompertz B and M values, as well as the maximum

population density (MPD), were generated using the SAS General Linear Model procedure (SAS 1989). Separate models were developed for the aerobic and anaerobic data.

RESULTS AND DISCUSSION

Prior to the development of response surface models, two sets of analyses were performed to assess which of the Gompertz parameters would be modeled. First, a series of supplemental experiments were performed to evaluate the effect of inoculum size on the growth kinetics of *S. aureus*. Inoculum levels between 10^1 and 10^6 cfu/ml were employed in conjunction with BHI containing 0.5% NaCl and 0 μg/ml $NaNO_2$, with the cultures being incubated at 19, 28, 37, and 42C

TABLE 1.
EFFECT OF INOCULUM SIZE ON THE GROWTH KINETICS OF *STAPHYLOCOCCUS AUREUS* 196E CULTURED AEROBICALLY IN BRAIN HEART INFUSION BROTH (pH 7.2, 0.5% NACL) AT DIFFERENT TEMPERATURES

Temp (C)	Inoculum Size (cfu/ml)	n[a]	Generation Time (h)	Lag Phase Duration (h)	Maximum Population Density (Log[cfu/ml])
42	2.53 (0.03)[b]	2	0.303 (0.013)	1.40 (0.13)	9.04 (0.00)
	3.51 (0.05)	2	0.322 (0.031)	1.70 (0.21)	9.16 (0.02)
	4.42 (0.01)	2	0.304 (0.005)	1.26 (0.03)	9.31 (0.03)
	5.43 (0.00)	2	0.290 (0.002)	1.29 (0.05)	9.48 (0.00)
37	2.86 (0.07)	2	0.285 (0.002)	2.13 (0.11)	9.29 (0.00)
	3.49 (0.10)	2	0.278 (0.002)	1.74 (0.20)	9.44 (0.02)
	4.63 (0.02)	2	0.269 (0.001)	1.83 (0.03)	9.44 (0.01)
	5.38 (0.00)	2	0.302 (0.014)	1.27 (0.06)	9.41 (0.00)
28	1.31 (0.01)	3	0.591 (0.012)	1.66 (0.60)	9.95 (0.10)
	2.31 (0.21)	3	0.607 (0.013)	1.43 (0.38)	10.06 (0.21)
	2.78 (0.08)	3	0.573 (0.001)	1.69 (0.27)	9.62 (0.13)
	3.60 (0.11)	4	0.618 (0.072)	1.19 (0.49)	9.77 (0.27)
	4.69 (0.13)	3	0.714 (0.047)	0.77 (0.09)	9.77 (0.34)
	5.62 —	1	0.790 —	0.17 —	9.51 —
19	2.64 (0.02)	2	1.738 (0.002)	7.70 (0.13)	8.49 (0.05)
	3.49 (0.01)	2	2.030 (0.241)	5.49 (1.86)	8.48 (0.04)
	4.48 (0.02)	2	2.548 (0.013)	1.86 (0.25)	8.95 (0.04)
	5.43 (0.03)	2	2.111 (0.138)	4.63 (0.76)	9.07 (0.08)

[a]n = Number of replicate cultures.

[b]Values represent: Mean (Standard Deviation).

(Table 1). Little, if any effect on the growth kinetics of *S. aureus* could be attributed to differences in inoculum levels. This is similar to observations with vegetative cells of a number of other Gram-positive and Gram-negative foodborne pathogens including *Salmonella* spp. (Gibson *et al.* 1988), *Listeria monocytogenes* (Buchanan and Phillips 1990), *Aeromonas hydrophila* (Palumbo *et al.* 1991, 1992), *Shigella flexneri* (Zaika *et al.* 1992), and *Escherichia coli* O157:H7 (Buchanan *et al.* 1993). While the cultural conditions used to assess the effect of inoculum size were close to optimal, recent studies with *E. coli* have indicated that inoculum levels have little impact on growth kinetics even when multiple cultural conditions are suboptimal (Buchanan *et al.* 1993). Like the previous researchers, identification of this characteristic allowed exclusion of the Gompertz A term from consideration from modeling.

The second supplemental analysis was an evaluation of the effect of the independent variables on the MPD achieved by *S. aureus* cultures to determine the extent of independence. Previous studies have suggested that for many foodborne pathogens, the MPD reached by an organism is largely independent of variables such as temperature, pH, water activity (Gibson *et al.* 1988; Buchanan and Phillips 1990; Palumbo *et al.* 1991; Zaika *et al.* 1992; Buchanan *et al.* 1993). Only when the values for independent variables approached the limits that sup-

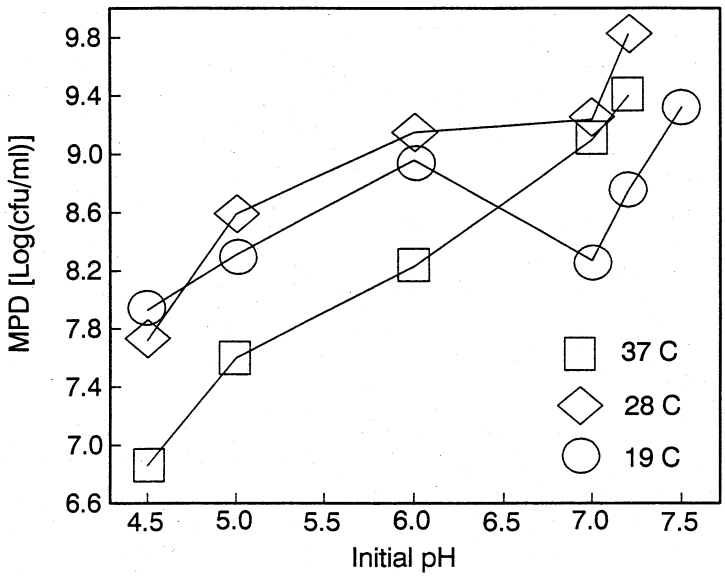

FIG. 1. EXAMPLES OF THE EFFECT OF PH AND TEMPERATURE ON THE MAXIMUM POPULATION DENSITY (MPD) ACHIEVED BY *STAPHYLOCOCCUS AUREUS* 196E CULTURED IN BRAIN HEART INFUSION BROTH (0.5% NACL, 0 μG/ML $NANO_2$)

TABLE 2.
COMPARISON OF OBSERVED vs. PREDICTED GROWTH KINETICS VALUES FOR AEROBIC CULTURES OF *STAPHYLOCOCCUS AUREUS* 196E

					Observed			Predicted[a]		
Temp (C)	NaCl (%)	$NaNO_2$ (μg/ml)	pH	n[b]	GT (h)	LPD (h)	MPD (Log[cfu/ml])	GT (h)	LPD (h)	MPD (Log[cfu/ml])
45	8.5	100	4.8	2	No Growth			1.5	25.5	6.5
45	8.5	0	6.7	2	1.0	18.9	7.2	0.5	6.6	8.0
45	8.5	100	6.7	2	1.0	16.0	6.7	0.8	7.2	7.5
45	8.5	100	8.4	2	No Growth			0.7	24.5	8.0
45	8.5	200	6.6	2	0.6	19.2	7.0	0.6	13.7	7.4
45	16.5	100	6.7	2	No Growth			1.0	9.3	5.9
42	0.5	0	4.5	2	No Growth			1.1	12.0	6.8
42	0.5	0	5.0	3	1.0	2.1	7.1	0.8	5.2	7.3
42	0.5	100	5.0	1	2.0	22.5	7.3	1.0	16.9	7.2
42	0.5	0	6.0	3	0.4	2.1	8.2	0.4	1.5	8.2
42	0.5	100	6.0	1	1.0	1.6	7.7	0.6	4.2	8.0
42	0.5	0	7.0	3	0.4	0.9	9.0	0.3	1.5	8.9
42	0.5	100	7.0	1	0.3	2.1	9.2	0.4	2.1	8.8
42	0.5	0	7.2	8	0.3	1.4	9.2	0.3	1.7	9.1
42	4.5	0	5.0	1	1.1	7.9	7.3	1.0	7.6	7.4
42	4.5	100	5.0	1	0.9	10.4	7.5	1.2	19.4	7.2
42	4.5	0	6.0	1	0.5	2.3	8.4	0.6	3.1	8.2
42	4.5	100	6.0	1	0.5	2.0	8.8	0.8	6.0	7.9
42	4.5	0	7.0	1	0.4	2.6	9.1	0.4	3.7	8.9
42	4.5	100	7.0	1	0.5	2.4	9.4	0.6	4.1	8.5
42	8.5	0	5.0	1	1.2	12.1	7.3	1.2	9.6	7.3
42	8.5	100	5.0	1	3.7	19.1	7.6	1.4	19.7	6.9
42	8.5	0	6.0	1	0.6	4.0	7.7	0.8	5.3	7.9
42	8.5	100	6.0	1	0.8	3.6	7.6	1.0	7.5	7.5
42	8.5	0	7.0	1	0.6	3.9	8.1	0.6	7.4	8.5
42	8.5	100	7.0	1	0.8	4.5	7.7	0.8	6.7	8.0
42	12.5	0	5.0	1	1.8	8.2	7.2	1.4	10.7	6.9
42	12.5	100	5.0	1	No Growth			1.6	17.6	6.4
42	12.5	0	6.0	1	1.1	8.3	7.7	0.9	7.4	7.4
42	12.5	100	6.0	1	0.9	6.9	7.6	1.2	8.2	6.9
42	12.5	0	7.0	1	0.7	6.8	8.0	0.7	12.1	7.8
42	12.5	100	7.0	1	1.3	7.2	7.7	1.0	9.1	7.2
37	0.5	0	4.5	2	2.6	7.7	6.9	1.2	13.7	7.3
37	0.5	0	5.0	2	1.2	4.8	7.7	0.8	5.6	7.8
37	0.5	0	6.0	2	0.5	1.2	8.2	0.5	1.3	8.6
37	0.5	0	7.0	2	0.3	1.7	9.1	0.3	1.1	9.3
37	0.5	0	7.2	8	0.3	1.7	9.4	0.3	1.3	9.4
37	4.5	50	5.6	2	0.5	9.0	8.8	1.0	5.3	8.2
37	4.5	150	5.7	3	0.3	13.6	8.2	0.9	13.9	8.2
37	4.5	50	7.5	3	0.8	1.2	8.8	0.6	4.1	9.2
37	4.5	150	7.5	3	0.8	5.8	8.7	0.7	5.1	9.0
37	12.5	50	5.7	3	2.0	8.2	7.4	1.8	7.9	7.4
37	12.5	150	5.8	3	1.8	7.8	7.3	1.5	13.2	7.1
37	12.5	50	7.4	3	2.0	13.2	8.2	1.3	14.0	7.8

port growth was there any depression of the MPD. However, when a similar evaluation was performed with *S. aureus* data, it became apparent the MPD was affected by cultural factors, particularly pH. For example, Fig. 1 depicts the effect of pH on the MPD reached by *S. aureus* cultures (aerobic, 0.5% NaCl, 0 μg/ml $NaNO_2$) incubated at various temperatures. Based on these observations, it was concluded that MPD, along with the Gompertz B and M values, had to be modeled. By modeling MPD, the Gompertz C value could be calculated using the relationship, MPD = C + A.

TABLE 2 (*continued*)

Temp (C)	NaCl (%)	$NaNO_2$ (μg/ml)	pH	n[b]	Observed GT (h)	Observed LPD (h)	Observed MPD (Log[cfu/ml])	Predicted[a] GT (h)	Predicted[a] LPD (h)	Predicted[a] MPD (Log[cfu/ml])
37	12.5	150	7.4	2	2.3	7.2	8.4	1.3	11.0	7.4
28	0.5	0	4.5	2	0.5	73.4	7.7	2.0	29.1	7.9
28	0.5	0	5.0	2	1.1	17.3	8.6	1.4	11.2	8.3
28	0.5	0	6.0	2	0.6	2.6	9.2	0.8	1.9	9.0
28	0.5	100	6.6	3	1.2	1.4	9.6	0.9	1.5	9.2
28	0.5	0	7.0	2	0.6	2.2	9.2	0.6	1.2	9.5
28	0.5	0	7.2	17	0.6	1.3	9.8	0.5	1.4	9.6
28	8.5	100	4.8	3	No Growth			4.8	65.4	7.9
28	8.5	0	6.7	3	3.0	3.5	9.0	2.1	9.2	9.0
28	8.5	100	6.8	3	2.8	7.3	8.4	2.7	5.0	8.6
28	8.5	200	6.8	3	3.0	10.5	8.4	1.9	14.7	8.5
28	16.5	100	8.4	3	2.1	35.3	8.4	2.6	25.7	8.5
28	0.5	100	6.7	3	5.0	15.5	5.5	7.1	9.0	6.8
19	0.5	0	4.5	2	8.5	56.5	7.9	6.0	117.4	7.9
19	0.5	0	5.0	2	3.5	9.4	8.3	4.4	45.1	8.3
19	0.5	0	6.0	2	1.8	9.8	9.0	2.6	7.6	8.8
19	0.5	0	7.0	2	1.7	7.2	8.3	1.8	3.3	9.1
19	0.5	0	7.2	8	2.1	4.9	8.3	1.7	3.7	9.1
19	0.5	0	7.5	1	2.1	2.9	9.3	1.6	4.9	9.2
19	0.5	200	7.5	1	2.1	2.2	9.5	2.0	7.9	9.3
19	4.5	0	7.5	1	3.2	5.3	9.5	6.7	21.6	9.1
19	4.5	50	5.8	2	5.1	5.9	8.0	6.4	60.0	8.7
19	4.5	50	7.6	2	5.3	4.0	8.9	4.7	8.8	8.9
19	4.5	150	5.8	2	6.9	88.5	8.0	6.4	60.0	8.7
19	4.5	150	7.6	2	4.6	9.1	8.7	5.0	5.2	8.8
19	4.5	200	7.5	1	3.6	11.3	9.6	4.1	14.3	8.8
19	8.5	0	7.5	1	5.7	31.4	8.9	8.3	55.0	8.7
19	8.5	200	7.5	1	6.3	44.4	8.9	8.2	21.9	8.2
19	12.5	0	7.5	1	No Growth			17.6	133.5	8.0
19	12.5	50	5.9	2	38.6	77.1	7.7	26.4	20.6	7.9
19	12.5	50	7.5	2	29.9	148.0	8.6	21.6	58.3	7.7
19	12.5	150	5.9	2	No Growth			21.7	56.6	7.6
19	12.5	150	7.5	2	28.2	144.2	8.0	20.6	14.7	7.3
19	16.5	0	7.5	1	No Growth			36.7	276.3	7.1
19	16.5	200	7.5	1	No Growth			31.2	27.0	6.2
15	0.5	0	4.5	2	No Growth			11.6	265.9	7.8
15	0.5	0	5.0	2	No Growth			8.5	102.8	8.1
15	0.5	0	6.0	2	5.6	28.4	8.6	5.1	18.5	8.5
15	0.5	0	7.0	2	3.1	3.2	8.8	3.6	7.7	8.7
12	0.5	0	4.5	2	No Growth			20.5	530.0	7.6
12	0.5	0	5.0	2	14.5	232.6	8.2	15.1	206.3	7.9
12	0.5	0	6.0	2	12.7	36.5	8.4	9.2	39.1	8.2
12	0.5	0	7.0	2	4.7	28.3	8.2	6.5	16.0	8.4

[a]A Gompertz A-value of 3.00 was assumed.

[b]Number of replicate cultures.

A total of 194 aerobic and 189 anaerobic growth curves were generated, representing 90 and 114 different combinations, respectively, of the four variables. The mean GT and LPD values are presented in Tables 2 and 3. Space constraints preclude the inclusion of all of the Gompertz terms and derived kinetic values for each of the replicates; however, these data are available to interested parties upon request. General observations on the cultural characteristics of *S. aureus* that support findings by other investigators (Smith *et al.* 1983) include (1) enhanced growth in the presence of oxygen, (2) ability to grow in the presence of elevated

TABLE 3.
COMPARISON OF OBSERVED VS. PREDICTED GROWTH KINETICS VALUES FOR ANAEROBIC CULTURES OF *STAPHYLOCOCCUS AUREUS* 196E

					Observed			Predicted[a]		
Temp (C)	NaCl (%)	Na NO_2 µg/ml	pH	n[b]	GT (h)	LPD (h)	MPD (Log[cfu/ml])	GT (h)	LPD (h)	MPD (Log[cfu/ml])
42	0.5	0	5.3	3	0.7	2.4	6.1	1.0	3.2	6.6
42	0.5	0	6.5	3	0.4	2.4	8.1	0.4	1.0	7.8
42	0.5	0	7.8	3	0.3	1.9	8.5	0.4	1.9	8.3
42	5.5	0	5.3	1	0.6	3.8	7.5	0.7	3.2	7.2
42	5.5	100	5.3	3		No Growth		0.7	5.6	7.3
42	5.5	100	6.5	3	0.4	3.0	8.5	0.4	2.3	8.4
42	5.5	100	7.8	3	0.5	2.0	8.5	0.4	4.1	8.6
42	12.5	0	5.5	1	1.6	4.2	7.3	1.1	4.0	7.3
42	12.5	0	6.5	1	2.2	1.2	7.6	1.0	3.5	7.7
42	12.5	50	6.5	1	1.7	1.3	8.0	1.0	3.9	7.9
42	12.5	0	7.5	1	0.6	6.1	6.7	1.6	9.2	7.6
42	12.5	0	8.5	1		No Growth		4.1	49.1	7.0
42	12.5	200	8.5	1		No Growth		2.0	38.8	7.5
42	16.5	0	6.0	1	2.0	6.8	7.1	2.6	5.7	6.9
42	16.5	50	6.0	1	2.6	7.2	7.2	2.6	6.3	7.0
42	16.5	0	7.0	1	6.1	9.3	7.4	3.9	11.6	6.8
42	16.5	100	7.0	1		No Growth		3.3	12.2	7.1
42	16.5	0	7.5	1		No Growth		5.8	23.9	6.6
42	16.5	200	7.5	1		No Growth		3.0	24.8	7.2
37	0.5	0	5.3	3	1.2	1.0	7.5	1.1	3.7	7.0
37	0.5	0	7.8	3	0.4	1.7	8.8	0.4	1.6	8.7
37	0.5	0	8.5	1		No Growth		0.5	4.4	8.6
37	3.0	0	5.3	1	0.8	4.8	7.4	0.8	3.6	7.3
37	3.0	50	5.3	3		No Growth		0.9	5.0	7.3
37	3.0	0	6.0	1	0.5	1.0	8.2	0.5	1.5	8.1
37	3.0	50	6.0	1	0.4	2.5	8.2	0.5	2.0	8.1
37	3.0	50	7.8	3	0.3	2.6	8.7	0.4	2.5	8.9
37	3.0	150	5.3	3		No Growth		0.9	9.6	7.3
37	3.0	150	7.8	3	0.3	4.9	8.9	0.4	3.5	8.9
37	8.0	50	5.3	3		No Growth		0.9	5.5	7.7
37	8.0	0	6.0	1	0.8	5.7	7.6	0.6	2.2	8.3
37	8.0	50	6.0	1	3.6	11.0	8.9	0.6	2.7	8.3
37	8.0	50	7.8	3	0.2	9.3	8.6	0.8	5.2	8.6
37	8.0	150	5.3	3		No Growth		0.9	9.5	7.7
37	8.0	150	7.8	3	0.5	4.0	8.6	0.6	6.3	8.7
37	12.5	0	6.5	1	1.1	4.4	8.0	1.2	3.8	7.9
37	16.5	0	6.0	1	3.3	2.4	7.8	3.2	6.9	7.0
37	16.5	100	6.0	1	7.2	22.0	6.9	3.2	9.0	7.3
37	16.5	100	6.5	1	1.3	16.5	7.3	3.6	9.2	7.3
37	16.5	0	7.0	1	3.7	2.9	5.5	4.7	12.3	7.0
37	16.5	100	7.0	1	2.1	8.0	7.9	4.4	12.8	7.2
37	16.5	200	7.0	1	2.5	7.9	7.6	3.2	17.5	7.4
37	16.5	200	7.5	1	4.1	13.5	7.4	4.3	27.1	7.2
37	16.5	0	8.5	1		No Growth		22.0	159.5	6.0
28	0.5	0	4.0	2		No Growth		10.8	120.8	5.6
28	0.5	0	6.5	3	0.9	3.3	8.5	0.7	1.7	8.6
28	0.5	100	6.5	3	0.7	2.3	8.5	0.9	3.5	8.4
28	0.5	0	9.0	3	1.5	4.9	8.5	1.2	12.6	8.6
28	5.5	100	4.0	3		No Growth		8.8	173.0	6.2
28	5.5	0	5.5	1	0.9	7.2	7.7	1.2	7.3	8.1
28	5.5	50	5.5	1	4.4	151.6	7.3	1.5	9.4	8.1
28	5.5	0	6.5	3	0.7	4.9	8.6	0.8	3.2	8.8
28	5.5	100	6.5	3	1.0	4.9	8.5	1.0	5.0	8.7
28	5.5	200	6.5	3	0.8	10.2	8.4	0.9	8.9	8.6
28	5.5	0	7.5	1	0.5	6.4	8.6	0.9	4.1	9.0
28	5.5	200	7.5	1	0.8	15.4	8.7	0.9	8.1	8.8
28	5.5	100	9.0	5	2.5	66.9	8.2	2.5	32.1	8.1
28	10.5	100	6.5	3	2.5	8.9	8.6	1.9	8.7	8.4
28	12.5	0	6.0	1	3.5	23.1	7.9	2.5	10.4	7.9
28	12.5	100	6.0	1	2.3	9,8	8.6	3.0	14.2	7.9

TABLE 3 (*continued*)

Temp (C)	NaCl (%)	Na NO_2 µg/ml	pH	n[b]	Observed GT (h)	Observed LPD (h)	Observed MPD (Log[cfu/ml])	Predicted[a] GT (h)	Predicted[a] LPD (h)	Predicted[a] MPD (Log[cfu/ml])
28	16.5	0	5.0	1	4.7	33.7	6.3	8.6	44.3	6.7
28	16.5	50	5.0	1		No Growth		10.0	51.6	6.7
28	16.5	0	6.0	1	7.3	23.2	7.0	7.5	21.1	7.0
28	16.5	0	7.5	1	15.3	15.5	7.2	15.6	50.4	6.6
28	16.5	200	7.5	1	14.3	221.6	7.3	13.3	56.1	6.8
19	3.0	0	5.3	1	2.1	8.8	7.9	5.2	50.9	7.5
19	3.0	50	5.3	3		No Growth		6.7	67.7	7.4
19	3.0	0	6.0	1	2.0	7.5	8.4	2.9	18.8	8.2
19	3.0	50	6.0	1	0.5	19.6	8.3	3.7	23.8	8.1
19	3.0	0	7.0	1	1.7	8.6	8.4	2.2	10.0	8.7
19	3.0	200	7.0	1	0.8	18.3	8.4	3.1	26.1	8.1
19	3.0	50	7.8	3	3.7	4.7	8.3	2.8	13.6	8.6
19	3.0	150	5.3	3		No Growth		9.1	125.8	7.1
19	3.0	150	7.8	3	2.4	9.2	8.2	3.1	19.6	8.4
19	8.0	0	5.3	1	2.8	21.1	6.4	6.2	68.2	7.6
19	8.0	50	5.3	3		No Growth		7.8	86.2	7.5
19	8.0	0	6.0	1	3.7	15.3	7.9	4.2	31.2	8.1
19	8.0	0	7.0	1	2.6	14.4	7.7	3.9	22.5	8.3
19	8.0	50	7.8	3	4.5	30.7	8.3	5.9	34.3	8.1
19	8.0	150	5.3	3		No Growth		10.4	145.7	7.3
19	8.0	150	7.8	3	5.7	30.4	8.1	6.6	42.5	7.9
19	12.5	0	6.0	1	11.7	37.1	8.3	9.7	63.1	7.4
19	12.5	100	6.0	1	2.4	119.1	6.8	13.6	84.5	7.4
19	12.5	0	7.0	1	16.2	44.4	7.3	11.2	57.4	7.4
19	12.5	200	7.0	1	15.1	41.0	6.5	14.8	89.9	7.2
19	16.5	0	5.5	1	24.1	251.7	6.5	32.1	191.0	6.4
19	16.5	50	5.5	1		No Growth		39.4	216.0	6.5
19	16.5	0	6.0	1	51.6	80.1	7.2	31.5	143.7	6.5
19	16.5	0	6.5	1	85.7	455.7	6.2	35.1	133.8	6.5
19	16.5	50	6.5	1	48.4	165.9	6.9	41.6	135.1	6.5
19	16.5	200	6.5	1		No Growth		47.8	204.0	6.4
19	16.5	0	7.0	1	34.3	120.1	7.0	44.5	159.0	6.3
19	16.5	50	7.5	1	49.8	216.4	4.8	73.4	226.1	6.1
19	16.5	0	8.0	1		No Growth		106.0	462.4	5.8
19	16.5	200	8.0	1		No Growth		116.1	429.9	5.8
12	0.5	0	5.3	3		No Growth		22.4	281.7	6.9
12	0.5	0	6.0	1	9.1	341.2	6.7	11.4	93.8	7.6
12	0.5	0	6.5	1	14.6	13.5	7.6	8.5	55.6	7.9
12	0.5	200	6.5	1	15.4	547.3	7.7	17.5	170.8	7.1
12	0.5	0	7.0	1	9.7	326.2	6.9	7.2	40.6	8.2
12	0.5	200	7.0	1	2.6	420.0	6.5	13.8	112.2	7.3
12	0.5	0	7.8	2	5.1	84.6	8.4	7.1	40.0	8.2
12	4.0	0	6.0	1	12.1	62.7	7.7	11.6	118.6	7.7
12	4.0	100	6.0	1		No Growth		19.2	193.3	7.4
12	4.0	0	7.0	1	8.7	340.0	7.3	8.7	64.0	8.1
12	4.0	200	7.0	1	52.1	36.4	7.3	16.1	145.5	7.4
12	5.5	100	5.3	3		No Growth		36.4	579.0	6.9
12	5.5	0	6.5	1	22.8	433.7	6.7	10.9	94.9	7.9
12	5.5	0	7.5	1	11.7	438.0	8.0	11.3	85.5	7.9
12	5.5	100	7.8	3		No Growth		17.7	115.3	7.6
12	8.0	0	6.5	1	8.7	691.7	6.2	15.4	137.9	7.6
12	8.0	50	6.5	1		No Growth		19.8	160.1	7.5
12	8.0	0	7.5	1	5.5	679.7	4.7	18.0	141.2	7.6
12	8.0	100	7.5	1		No Growth		26.2	167.4	7.4

[a]A Gompertz A-value of 3.00 was assumed.

[b]Number of replicate cultures.

TABLE 4.
QUADRATIC RESPONSE SURFACE MODELS FOR THE EFFECTS OF TEMPERATURE, pH, SODIUM CHLORIDE AND SODIUM NITRITE ON THE AEROBIC AND ANAEROBIC GROWTH OF *STAPHYLOCOCCUS AUREUS* 196E

T = C (12 - 42)
P = Initial pH (4.5 - 8.4)
S = NaCl (% W/V) (0.5 - 16.5) = g/L ÷ 10
N = $NaNO_2$ (μg/ml) (0 - 200)
MPD = Maximum population density [Log(cfu/ml)]

AEROBIC

$$LN(B) = -10.8812 + 0.2551*T + 1.0648*P - 0.2653*S + 0.000379*N - 0.00133*TP + 0.00516*TS - 0.0000335*TN - 0.00723*PS - 0.000845*PN + 0.000199*SN - 0.00273*T^2 - 0.0563*P^2 + 0.00308*S^2 + 0.0000270*N^2$$

Adjusted $R^2 = 0.92^a$

$$LN(M) = 24.1321 - 0.3667*T - 4.5705*P + 0.0536*S + 0.0155*N + 0.0110*TP - 0.00324*TS + 0.000103*TN + 0.0369*PS - 0.00241*PN - 0.000449*SN + 0.00366*T^2 + 0.301*P^2 - 0.00372*S^2 + 0.0000117*N^2$$

Adjusted $R^2 = 0.94$

$$LN(MPD) = 1.4074 + 0.00765*T + 0.1588*P + 0.0330*S + 0.000265*N + 0.00241*TP - 0.0000980*TS - 0.00000501*TN - 0.00355*PS - 0.0000613*PN - 0.0000483*SN - 0.000413*T^2 - 0.0129*P^2 - 0.00122*S^2 + 0.00000199*N^2$$

Adjusted $R^2 = 0.79$

ANAEROBIC

$$LN(B) = -17.4388 + 0.2878*T + 2.9084*P + 0.2414*S - 0.0115*N - 0.00353*TP + 0.00130*TS + 0.000144*TN - 0.0381*PS + 0.000673*PN + 0.0000116*SN - 0.00303*T^2 - 0.1929*P^2 - 0.00942*S^2 + 0.0000136*N^2$$

Adjusted $R^2 = 0.92$

$$LN(M) = 27.5191 - 0.4169*T - 5.2023*P - 0.2363*S + 0.0133*N + 0.0128*TP - 0.00158*TS - 0.0000708*TN + 0.0447*PS - 0.00188*PN - 0.000133*SN + 0.00406*T^2 + 0.3401*P^2 + 0.00752*S^2 - 0.00000152*N^2$$

Adjusted $R^2 = 0.93$

TABLE 4. (*Continued*)

$$LN(MPD) = 0.0290 + 0.0151*T + 0.5046*P + 0.0468*S - 0.000852*N + 0.000789*TP + 0.000382*TS + 0.0000181*TN - 0.00651*PS + 0.0000301*PN + 0.0000245*SN - 0.000389*T^2 - 0.0338*P^2 - 0.00165*S^2 - 0.000000651*N^2$$

Adjusted $R^2 = 0.80$

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[a]Adjusted $R^2 = R^2/\text{Max } R^2$ (Draper and Smith, 1981)

levels of sodium chloride, and (3) dependence of the bacteriostatic activity of sodium nitrite on pH and oxygen availability.

Quadratic and cubic models in conjunction with several transformations of the data for Gompertz B and M terms and MPD were evaluated. Since oxygen content was a qualitative variable, (i.e., aerobic versus anaerobic), separate models were developed for the two test conditions. Overall, the best agreement between observed and predicted values was achieved using quadratic models that employed a natural logarithm transformation (Table 4). Use of cubic models did not enhance the agreement between observed and predicted values, and accordingly, the simpler quadratic models were selected. The adjusted R^2 values for the B and M terms indicated a good fit between the models and the observed values. The adjusted R^2 values for the MPD models indicated a poorer fit than that achieved for B and M terms. However, comparison of predicted and observed values indicated that the correlations were sufficient to be useful. Comparisons of observed versus predicted values for GT, LPD, and MPD (Table 2 and 3) and times to a 1000-fold increase (Fig. 2) indicated that the models provide reasonable estimates of the effects and interactions of the four variables on the growth kinetics of *S. aureus*. The predicted values were calculated assuming a Gompertz A-value of 300; the approximate inoculum level used experimentally. Since the growth kinetics were independent of inoculum size, growth curves can be corrected for other inoculum levels using the approach of Buchanan (1991). Garthright (1991) suggested that the direct modeling of GT, LPD, and MPD may offer advantages over the modeling of the Gompertz terms. The latter approach was used in the current study to have results comparable to earlier studies (Buchanan and Phillips 1990; Palumbo *et al.* 1991, 1992; Zaika *et al.* 1992; Buchanan *et al.* 1993). However, the data sets are currently being reanalyzed to evaluate the hypothesis of Garthright (1991).

The models were particularly effective when none of the growth parameters was limiting. Because a natural logarithm transformation excludes no-growth data (i.e., the logarithm of 0.0 is undefined), these models tended to underestimate the impact of adverse conditions. However the use of an alternative transformation (i.e., square root transformation) that stabilized variance but allowed in-

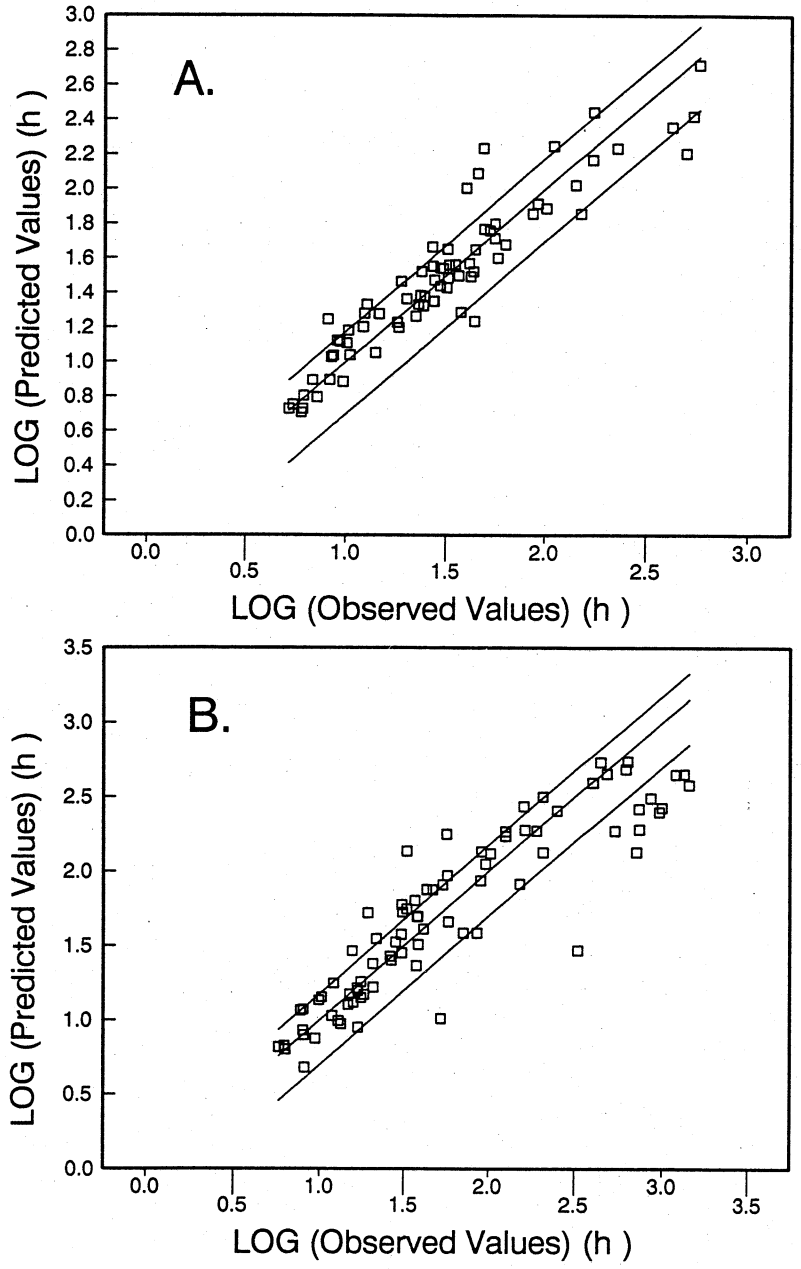

FIG. 2. COMPARISON OF PREDICTIONS OF TIMES NEEDED FOR THE POPULATION DENSITY OF *STAPHYLOCOCCUS AUREUS* 196E TO INCREASE 1000-FOLD VERSUS VALUES CALCULATED FROM EXPERIMENTAL DATA FOR AEROBIC (A) AND ANAEROBIC (B) CULTURES

Variable combinations that did not support growth were excluded.

corporation of no-growth data yielded models that overestimated the effectiveness of limiting growth conditions. Future research to enhance the precision of the models will target the acquisition of additional growth data at variable combinations that are limiting but still support the growth of the organism.

The F-values for the models were evaluated to assess the relative importance of the variables and their interactions (Table 5). In the aerobic cultures, temperature, salt content and pH accounted for the majority of the effects. The variables were largely independent, based on the small F-values associated with

TABLE 5.
F-VALUES FOR INDEPENDENT VARIABLES AND THEIR CROSS PRODUCTS FOR THE QUADRATIC MODELS

	AEROBIC			ANAEROBIC		
	B	M	MPD	B	M	MPD
T	416.0[a]	528.0[a]	21.4[a]	473.6[a]	654.2[a]	0.0
P	32.5[a]	179.2[a]	81.2[a]	12.4[a]	1.7	82.2[a]
S	119.5[a]	334.7[a]	105.7[a]	141.1[a]	187.1[a]	31.0[a]
N	3.1	24.8[a]	0.8	4.4[b]	0.1	7.4[b]
T*P	0.3	12.7[a]	4.7[b]	5.4[a]	3.1	18.5[a]
T*S	8.7[b]	0.5	1.1	1.8	3.8	11.0[b]
T*N	0.1	2.1	2.1	7.6[b]	6.3[b]	12.1[a]
P*S	3.0	41.7[a]	11.6[a]	2.0[b]	1.1	2.9
P*N	1.0	0.0	1.0	1.1	6.6[b]	5.1[b]
S*N	7.9[b]	22.3[a]	4.5[b]	2.1	0.9	1.2
T^2	20.8[a]	64.9[a]	20.0[a]	16.7[a]	31.2[a]	13.9[a]
P^2	1.7	45.5[a]	1.5	17.0[a]	61.9[a]	31.0[a]
S^2	0.6	3.7	9.0[b]	18.0[a]	16.3[a]	39.6[a]
N^2	2.9	1.1	0.8	1.0	0.0	0.1

[a] $P \leq 0.001$.

[b] $0.050 > P > 0.001$.

TABLE 6.
COMPARISON OF GROWTH KINETICS DATA REPORTED FOR *STAPHYLOCOCCUS AUREUS* VERSUS THAT PREDICTED BY THE AEROBIC RESPONSE SURFACE MODEL

						Observed		Predicted		
Product	Temp (C)	pH	^{a}w	NaCl (%)	NaNO2 (μg/ml)	LPD (h)	GT (h)	LPD (h)	GT (h)	Source
UHT Milk	16	6.5	0.93	(11.5)[a]	0	60.0	10.1	75.7	37.9	Broughall et al.(1983)
	16	6.5	0.95	(8.4)	0	14.4	4.1	45.3	20.1	
	20	6.5	0.98	(3.5)	0	6.0	1.3	7.3	3.6	
Raw Pastry	25	5.9	0.91	(14.6)	0	5.5	1.6	21.9	10.4	C. Adair (Personal
	30	5.9	0.91	(14.6)	0	7.0	1.2	14.0	4.5	Communication)
	37	5.9	0.91	(14.6)	0	2.0	0.7	9.3	1.7	
Steak &	13	5.9	0.98	(3.5)	0	50.4	8.7	62.7	17.3	C. Adair (Personal
Kidney Pie	21	5.9	0.98	(3.5)	0	8.0	2.3	10.8	3.9	Communication)
Mix	30	5.9	0.98	(3.5)	0	3.4	0.6	3.4	1.2	
	42	5.9	0.98	(3.5)	0	1.5	0.4	2.8	0.6	
Brain Heart	37	7.3	(0.99)	0.5	0	2.0	0.5	1.5	0.3	Buchanan & Solberg
Infusion	37	7.3	(0.99)	0.5	200	2.3	0.4	5.0	0.4	(1972)
	37	6.3	(0.99)	0.5	0	2.0	1.0	1.0	0.4	
	37	6.3	(0.99)	0.5	200	5.6	0.8	10.4	0.5	

[a]Parentheses indicate that value assumed based on the water activity or sodium chloride concentration indicated in the citation.

the cross-product terms. The F-values for the anaerobic cultures were similar except that there was a much larger proportion of the F-values attributable to the square terms for temperature, pH, and salt content. The MPD values for the anaerobic cultures were more strongly influenced by the interaction between primary variables.

The usefulness of the models for estimating the growth kinetics of *S. aureus* in food products was assessed by comparing the predicted values with reported data (Table 6). Considering the importance of *S. aureus* as a major cause of food poisoning, there is surprisingly little published quantitative growth kinetics data. There was reasonable agreement between predicted LPD and GT values and those observed with the three foods for which quantitative growth data was acquired. In general, the agreement was better at the higher storage temperatures and water activities. Some of the differential between the growth kinetics observed in specific foods and those predicted by the model may reflect strain differences. A more important factor may be the identity of the humectant used to modify the water activity of the foods. McMeekin *et al.* (1987) and Chandler and McMeekin (1989) demonstrated that the growth kinetics of *Staphylococcus xylosus* in relation to the combined effects of temperature and water activity were dependent on humectant identity. Humectant identity is known to influence both growth and enterotoxin production by *S. aureus* (Smith *et al.* 1983).

Overall, the current models provide a rapid means of acquiring "first estimates" of the growth of *S. aureus* in response to the interaction of five variables. These models have been incorporated into the latest version of the MFS Pathogen Modeling Program (Buchanan 1991), an application software which automates response surface models available for several foodborne pathogens. While the models provide reasonable estimates, it does appear that accuracy could be enhanced by the generation and incorporation of additional data, particularly in the regions where growth is limited. Development of expanded models that include the effect of humectant identity is an area for future research that would also enhance the usefulness of the current models.

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